## REMARKS

Claims 1-30 are pending. A "clean" claim set to be introduced in place of the original claims is provided above. A version showing changes made is attached for the Examiner's convenience. In addition, an appendix of pending claims is attached herein for the Examiner's convenience. The specification has been amended to correct a typographical error. The current office action does not make mention of the previous provisional rejection of claims 10-11 under the judicially created doctrine of obvious-type double patenting as being unpatentable over claims 1-2 of co-pending Application No. 09/425,633. In addition the current office action does not make mention of the previous rejections of claims 1-17 and 22-27 under 35 U.S.C. § 112, second paragraph. Applicants will assume that these rejections have been overcome.

**Rejections based under 35 U.S.C. § 103(a)**

Claims 1-4, 6-10, 12-17 and 22-27 are rejected under 35 U.S.C. § 103 as being unpatentable over Navot et al. (U.S. Patent No. 6,335,165 B1) and Walt et al. (U.S. Patent No. 6,327,410 B1).

Navot et al. is directed to methods and kits for characterizing G-C rich regions of a nucleic acid of interest. Method steps include modifying the guanine and cytosine residues by replacing them with residues complementary to adenine and thymine; amplifying the modified nucleic acid of interest and then subjecting the modified nucleic acid to sequencing which can include gel electrophoresis- free, pyrosequencing. Navot et al. does not teach or suggest the use of microspheres, randomly distributed on the surface of a substrate.

Walt et al. is directed to microspheres distributed on the surface of a substrate for detecting the presence of target analytes in a sample through the use of an optical encoding scheme.

In contrast, claim 1 of the present invention is drawn to a method of sequencing nucleic acid by detecting the release of pyrophosphate to determine the type of nucleotide added to a sequencing primer by providing hybridization complexes attached to microspheres randomly distributed on the surface of a substrate. Claim 10 is drawn to a method of sequencing a nucleic acid through the use of a capture probe covalently attached to a microsphere randomly distributed on a surface.

To establish a prima facie case of obviousness, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. In addition, the prior art reference (or references when combined) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, and not based on applicant's disclosure. *In re Vaeck*, 947 F2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991).

The Examiner states that it would have been obvious to one of ordinary skill in the art to use the microsheres randomly distributed on the surface of Walt et al. as the beads in the pyrosequencing of Navot et al. The Examiner goes on to state that Walt et al. expressly provides the motivation to do so by stating that the synthesis of nucleic acids is separated from their placement on the array and random distribution of beads is fast and inexpensive. Applicants respectfully disagree.

Applicants would like to respectfully point out that synthesis of nucleic acids being separated from their placement on the array, as the Examiner refers to as the express statement of motivation, is not equivalent to determining the DNA sequence. That is, synthesizing DNA in this context is distinct from sequencing a target sequence. The synthesis of nucleic acids and distribution of beads on an array does not suggest any combination with a pyrosequencing method to determine the sequence of a target sequence hybridized to the bead. Neither prior art reference, either alone or in combination, suggest the desirability of combining or modifying either prior art reference to reach the claims of the present invention, namely claims 1 and 10 from which all other claims depend. Neither prior art reference, alone or in combination, suggests the use of microspheres randomly distributed on a surface for sequencing a plurality of target nucleic acids by the methods of pyrosequencing (claim1) or a method of sequencing a nucleic acid through the use of a capture probe covalently attached to a microsphere randomly distributed on the surface of a substrate (claim 10).

Therefore, the requirement that there be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings has not been met. Accordingly, the Examiner has failed to make out a prima facie case of obviousness.

Claims 5 and 11 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Navot et al. and Walt et al. as applied to claims 1 and 10 above, and in further view of Balch (U.S. Patent No. 6,083,763).

The distinctions between Navot et al. and Walt et al. and the claims of the present invention are discussed above. As the Examiner points out, neither Navot et al. nor Walt et al. teach adapter probes.

Balch et al. is directed to a mutiplexed molecular analysis system of detecting target analytes within a sample through the use of "charged coupled device" (CCD) technology. Balch et al. does not teach microspsheres randomly distributed on the surface of a substrate nor does it teach the use of pyrosequencing of nucleic acid.

Claim 5 is drawn to a method of sequencing nucleic acid by detecting the release of pyrophosphate to determine the type of nucleotide added to a sequencing primer by providing hybridization complexes attached through the use of capture and adapter probes to microspheres randomly distributed on the surface of a substrate. Claim 11 is drawn to a method of sequencing a nucleic acid through the use of an adapter probe and an capture probe covalently attached to a microsphere randomly distributed on a surface.

As stated above, to establish a prima facie case of obviousness, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. The teaching or suggestion to make the claimed combination and the claim limitations must both be found in the prior art, and not based on applicant's disclosure. *In re Vaeck*, 947 F2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991).

The Examiner states that it would have been obvious to one of ordinary skill in the art to have used the adapter probes of Balch for the formation of primer-target complexes in the combined method of Navot et al. And Walt et al. The Examiner states that the motivation to do

so would have been that adapter probes delivered a unique binding domain for each site on an array. Applicants respectfully disagree.

Neither Navot et al., Walt et al. or Balch et al., alone or in combination, suggest the combination or modification of the prior art references in order to reach the claims of the present invention. None of the prior art references,alone or in combination, suggests modifying or combining the reference (or references) to reach a method of pyrosequencing nucleic acid on microspheres randomly distributed on the surface of a substrate, which are elements of claims 5 and 11.

The Examiner's attention in respectfully drawn to In re Lee, 61 USPQ2d 1430 (CA FC 2002). In this case, the Examiner rejected the claims under 35 U.S.C. §103 and stated that the required motivation "would be that the automatic demonstration mode is user friendly and it functions as a tutorial". Id at 1435. The Federal Circuit stated that "deficiencies of the cited references cannot be remedied by the Board's general conclusions about what is "basic knowledge" or "common sense"". The Board's finding must extend to all material facts and must be documented on the record, lest the "haze of so-called expertise" acquire insulation from accountability. "Common knowledge" and "common sense", even if assumed to derived from the agency's expertise, do not substitute for authority when the law requires authority." (citing In re Zurko, 59 USPQ2d 1693 (CA FC 2001); see Lee, 1434-1435).

In this case, the Examiner has essentially used impermissible hindsight and "common sense" to conclude that the combination of these two references leads to "a unique binding domain for each site on an array". This is legally incorrect under the Federal Circuit's analysis.

Therefore , the requirement that there be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings has not been met.

Applicants respectfully request the withdrawal of the rejection.

Claims 18-21 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Navot et al. and Walt et al. as applied to claims 1 and 10, in view of Nyren et al. (WO 98/13523).

The distinctions between Navot et al. and Walt et al. and the claims of the present invention are discussed above.

Nyren et al. discloses a method of sequencing DNA based on the detection of base incorporation by the release of pyrophosphate (pyrosequencing). Nyren et al. does not teach or suggest a method of pyrosequencing through the use of microspheres randomly distributed on the surface of a substrate. Furthermore, Nyren et al. does not disclose kits which contain all the reagents necessary for pyrosequencing on microspheres randomly distributed on the surface of a substrate.

Claims 18-21 are drawn to kits for nucleic acid sequencing comprising microspheres randomly distributed on the surface of a substrate, capture probes, extension enzymes and labeled dNTPs.

As mentioned above, in order to establish a prima facie case of obviousness, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings.

The Examiner states that it would have been obvious to one of ordinary skill in the art to add the kits of Nyren et al. to a kit and composition disclosed by Navot et al. and Walt et al. because kits are conventional in the filed of molecular biology and provide benefits of convenience and cost-effectiveness. Applicants respectfully disagree.

There is lacking any suggestion or motivation to modify the references or combine reference teachings. The mere fact that references can be combined or modified does not render the resultant combination obvious unless the prior art also suggests the desirability of the combination. *In re Mills*, 916 F 2d 680, 16 USPQ2d 1430 (Fed. Cir. 1990). There is no suggestion in either reference of modifying or combining to reach the claims of the present invention.

The Examiner's attention in respectfully drawn to In re Lee, Supra.

In this case, the Examiner has essentially used impermissible hindsight and "common sense" to conclude that the combination of these two references leads to "benefits of convenience and cost effectiveness". This is legally incorrect under the Federal Circuit's analysis.

There is no suggestion from any of the prior art references to reach claims which utilize kits for nucleic acid sequencing comprising microspheres randomly distributed on the surface of a substrate, capture probes, extension enzymes and labeled dNTPs. Therefore , the requirement that there be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings has not been met.

Applicants respectfully request the withdrawal of the rejections.

Applicants submit that the claims are now in condition for allowance and early notification to that effect is respectfully requested. If the Examiner feels there are further unresolved issues, the Examiner is respectfully requested to phone the undersigned at (415) 781-1989.

Respectfully submitted,

DORSEY & WHITNEY LLP

Dated: 7/5/02

David C. Lueth Reg. No. 44,685

for Robin M. Silva
Reg. No. 38,304
Filed under 37 C.F.R. § 1.34(a)

Four Embarcadero Center
Suite 3400
San Francisco, CA 94111-4187
Telephone: (415) 781-1989

## VERSION SHOWING CHANGES MADE

1. (amended) A method of sequencing a plurality of target nucleic acids each comprising a first domain and an adjacent second domain, said second domain comprising a plurality of target positions, said method comprising:

a) providing first and second hybridization complexes comprising first and second target sequences, respectively and first and second sequencing primers, respectively, that hybridize to the first domain of said first and second target sequences, respectively, said first and second hybridization complexes attached to first and second microspheres, respectively, randomly distributed on a surface of a substrate;

b) extending said first and second primers by the addition of a first nucleotide to[the] a first detection position using a first enzyme to form first and second extended primer, respectively; and

c) detecting the release of pyrophosphate (PPi) to determine the type of said first nucleotide added onto said first and second primers, respectively.

2. A method according to claim 1 wherein at least said first hybridization complex is covalently attached to said first microsphere.

3. A method according to claim 1 wherein at least said first sequencing primer is attached to said first microsphere.

4. A method according to claim 1 wherein said first and second hybridization complexes comprise said first and second target sequences, respectively, said first and second sequencing primers, respectively, and first and second capture probes, respectively, covalently attached to said first and second microspheres, respectively.

5. A method according to claim 1 wherein said first and second hybridization complexes comprise said first and second target sequences, respectively, said first and second sequencing primers, a first and second adapter probe, respectively, and first and second capture probes, respectively, covalently attached to said first and second microspheres.

6. A method according to claim 1 further comprising:

d) extending said first and second extended primers by the addition of a second nucleotide to a second detection position using said first enzyme; and

e) detecting the release of pyrophosphate (PPi) to determine the type of said second nucleotide added onto said first and second primers, respectively.

7. The method according to claim 1 wherein said PPi is detected by a method comprising:

a) contacting said PPi with a second enzyme that converts said PPi into ATP; and

b) detecting said ATP using a third enzyme.

8. A method according to claim 7 wherein said second enzyme is sulfurylase.

9. A method according to claim 7 wherein said third enzyme is luciferase.

10.(amended) A method of sequencing a target nucleic acid comprising a first domain and an adjacent second domain, said second domain comprising a plurality of target positions, said method comprising:

a) providing a hybridization complex comprising said target nucleic acid [sequence], and a capture probe covalently attached to a microsphere randomly distributed on a surface of a substrate; and

b) determining the identity of a plurality of bases at said target positions.

11. A method according to claim 10 wherein said hybridization complex comprises said capture probe, an adapter probe, and said target sequence.

12. A method according to claim 10 wherein said capture probe is a sequencing primer.

13. A method according to claim 10 wherein said determining comprises:

a) providing a sequencing primer hybridized to said second domain;

b) extending said primer by the addition of a first nucleotide to a first detection position using a first enzyme to form an extended primer;

c) detecting the release of pyrophosphate (PPi) to determine the type of said first nucleotide added onto said primer;

d) extending said primer by the addition of a second nucleotide to a second detection position using said enzyme; and

e) detecting the release of pyrophosphate (PPi) to determine the type of said first nucleotide added onto said primer.

14. The method according to claim 10 wherein said PPi is detected by a method comprising:

a) contacting said PPi with a second enzyme that converts said PPi into ATP; and

b) detecting said ATP using a third enzyme.

15. A method according to claim 14 wherein said second enzyme is sulfurylase.

16. A method according to claim 14 wherein said third enzyme is luciferase.

17. A method according to claim 10 wherein said determining comprises:

a) providing a sequence primer hybridized to said second domain;

b) extending said primer by the addition of a first protected nucleotide using a first enzyme to form an extended primer;

c) determining the identification of said first protected nucleotide;

d) removing the protection group;

e) adding a second protected nucleotide using said first enzyme; and

f) determining the identification of said second protected nucleotide.

18. A kit for nucleic acid sequencing comprising:

a) a composition comprising:

i) a substrate with a surface comprising discrete sites; and

ii) a population of microspheres randomly distributed on said sites;

wherein said microspheres comprise capture probes;

b) an extension enzyme; and

c) dNTPs.

19. A kit according to claim 18 further comprising:

d) a second enzyme for the conversion of pyrophosphate (PPi) to ATP; and

e) a third enzyme for the detection of ATP.

20. A kit according to claim 18 wherein said dNTPs are labeled.

21. A kit according to claim 20 wherein each dNTP comprises a different label.

22. The method according to claim 1, wherein said substrate comprises discrete sites and said first and second microspheres are randomly distributed on said sites.

23. The method according to claim 22, wherein said discrete sites are wells, and said first and second microspheres are randomly distributed in said wells.

24. The method according to claim 10, wherein said substrate comprises discrete sites and said microspheres are randomly distributed on said sites.

25. The method according to claim 10, wherein discrete sites are wells, and said microspheres are randomly distributed in said wells.

26. The method according to claim 1, 10, 22, 23, 24 or 25, wherein said substrate is a fiber optic bundle.

27. The method according to claim 1, 10, 22, 23, 24 or 25, wherein said substrate is selected from the group consisting of glass and plastic.

28. The kit according to claim 18, wherein discrete sites are wells.

29. The kit according to claim 18 or 28, wherein said substrate is a fiber optic bundle.

30. The kit according to claim 18 or 28, wherein said substrate is selected from the group consisting of glass and plastic.

## PENDING CLAIMS

1. A method of sequencing a plurality of target nucleic acids each comprising a first domain and an adjacent second domain, said second domain comprising a plurality of target positions, said method comprising:

a) providing first and second hybridization complexes comprising first and second target sequences, respectively and first and second sequencing primers, respectively, that hybridize to the first domain of said first and second target sequences, respectively, said first and second hybridization complexes attached to first and second microspheres, respectively, randomly distributed on a surface of a substrate;

b) extending said first and second primers by the addition of a first nucleotide to a first detection position using a first enzyme to form first and second extended primer, respectively; and

c) detecting the release of pyrophosphate (PPi) to determine the type of said first nucleotide added onto said first and second primers, respectively.

2. A method according to claim 1 wherein at least said first hybridization complex is covalently attached to said first microsphere.

3. A method according to claim 1 wherein at least said first sequencing primer is attached to said first microsphere.

4. A method according to claim 1 wherein said first and second hybridization complexes comprise said first and second target sequences, respectively, said first and second sequencing primers, respectively, and first and second capture probes, respectively, covalently attached to said first and second microspheres, respectively.

5. A method according to claim 1 wherein said first and second hybridization complexes comprise said first and second target sequences, respectively, said first and second sequencing primers, a first and second adapter probe, respectively, and first and second capture probes, respectively, covalently attached to said first and second microspheres.

6. A method according to claim 1 further comprising:

d) extending said first and second extended primers by the addition of a second nucleotide to a second detection position using said first enzyme; and

e) detecting the release of pyrophosphate (PPi) to determine the type of said second nucleotide added onto said first and second primers, respectively.

7. The method according to claim 1 wherein said PPi is detected by a method comprising:

a) contacting said PPi with a second enzyme that converts said PPi into ATP; and

b) detecting said ATP using a third enzyme.

8. A method according to claim 7 wherein said second enzyme is sulfurylase.

9. A method according to claim 7 wherein said third enzyme is luciferase.

10. A method of sequencing a target nucleic acid comprising a first domain and an adjacent second domain, said second domain comprising a plurality of target positions, said method comprising:

a) providing a hybridization complex comprising said target nucleic acid, and a capture probe covalently attached to a microsphere randomly distributed on a surface of a substrate; and

b) determining the identity of a plurality of bases at said target positions.

11. A method according to claim 10 wherein said hybridization complex comprises said capture probe, an adapter probe, and said target sequence.

12. A method according to claim 10 wherein said capture probe is a sequencing primer.

13. A method according to claim 10 wherein said determining comprises:

a) providing a sequencing primer hybridized to said second domain;

b) extending said primer by the addition of a first nucleotide to a first detection position using a first enzyme to form an extended primer;

c) detecting the release of pyrophosphate (PPi) to determine the type of said first nucleotide added onto said primer;

d) extending said primer by the addition of a second nucleotide to a second detection position using said enzyme; and

e) detecting the release of pyrophosphate (PPi) to determine the type of said first nucleotide added onto said primer.

14. The method according to claim 10 wherein said PPi is detected by a method comprising:

a) contacting said PPi with a second enzyme that converts said PPi into ATP; and

b) detecting said ATP using a third enzyme.

15. A method according to claim 14 wherein said second enzyme is sulfurylase.

16. A method according to claim 14 wherein said third enzyme is luciferase.

17. A method according to claim 10 wherein said determining comprises:

a) providing a sequence primer hybridized to said second domain;

b) extending said primer by the addition of a first protected nucleotide using a first enzyme to form an extended primer;

c) determining the identification of said first protected nucleotide;

d) removing the protection group;

e) adding a second protected nucleotide using said first enzyme; and

f) determining the identification of said second protected nucleotide.

18. A kit for nucleic acid sequencing comprising:

a) a composition comprising:

I) a substrate with a surface comprising discrete sites; and

ii) a population of microspheres randomly distributed on said sites;

wherein said microspheres comprise capture probes;

b) an extension enzyme; and

c) dNTPs.

19. A kit according to claim 18 further comprising:

d) a second enzyme for the conversion of pyrophosphate (PPi) to ATP; and

e) a third enzyme for the detection of ATP.

20. A kit according to claim 18 wherein said dNTPs are labeled.

21. A kit according to claim 20 wherein each dNTP comprises a different label.

22. The method according to claim 1, wherein said substrate comprises discrete sites and said first and second microspheres are randomly distributed on said sites.

23. The method according to claim 22, wherein said discrete sites are wells, and said first and second microspheres are randomly distributed in said wells.

24. The method according to claim 10, wherein said substrate comprises discrete sites and said microspheres are randomly distributed on said sites.

25. The method according to claim 10, wherein discrete sites are wells, and said microspheres are randomly distributed in said wells.

26. The method according to claim 1, 10, 22, 23, 24 or 25, wherein said substrate is a fiber optic bundle.

27. The method according to claim 1, 10, 22, 23, 24 or 25, wherein said substrate is selected from the group consisting of glass and plastic.

28. The kit according to claim 18, wherein discrete sites are wells.

29. The kit according to claim 18 or 28, wherein said substrate is a fiber optic bundle.

30. The kit according to claim 18 or 28, wherein said substrate is selected from the group consisting of glass and plastic.